Fundam. appl. Nematol., 1995, **18** (6), 513-522

Ecology and pathogenicity of the Hoplolaimidae (Nemata) from the sahelian zone of West Africa. 8. *Senegalonema sorghi* Germani, Luc & Baldwin, 1984 and comparison with *Rotylenchulus reniformis* Linford & Oliveira, 1940

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Accepted for publication 29 August 1994.

Summary – The geographical distribution, host plants, population dynamics and vertical distribution were studied for the nematode *Senegalonema sorghi* in Senegal. The observations of sorghum roots parasitized by *S. sorghi* showed the absence of gelatinous matrix and the presence of a shell around the mature females. The development of the female inside the roots induced the bursting and tearing of the cortical tissues of the roots. The factors influencing the multiplication rate and the effects of anhydrobiosis were studied in the laboratory for *S. sorghi* and *Rotylenchulus reniformis*. The results showed that the highest multiplication rates of both species were recorded at relatively low soil temperature and high soil moisture. Both species were able to enter anhydrobiosis during the dry season, with survival rates of 20-40 %. *S. sorghi* parasitized only wild and cropped cereals. During the dry season, it was under hydrobiotic conditions at depth in cropped soils and under anhydrobiosis in the upper layers of the soils under fallow. The restricted distribution of *R. reniformis* in the vegetable crops under irrigation might be explained by its narrow host range; all other ecological characteristics were the same as for *S. sorghi*.

Résumé – ***Écologie et nocuité des Hoplolaimidae (Nemata) de la zone sahélienne de l'Afrique de l'Ouest. 8.* Senegalonema sorghi *Germani, Luc & Baldwin, 1984 et comparaison avec* Rotylenchulus reniformis *Linford & Oliveira, 1940*** - La répartition géographique, les plantes hôtes, la dynamique et la répartition verticale des populations ont été étudiées au Sénégal pour *Senegalonema sorghi*. L'observation des racines de sorgho parasitées par *S. sorghi* montre l'absence de gangue gélatineuse et la présence d'une coque de nature inconnue chez les femelles matures dont le renflement provoque l'éclatement des tissus corticaux des racines. Les facteurs influençant le taux de multiplication et les effets de l'anhydrobiose ont été étudiés au laboratoire pour *S. sorghi* et *Rotylenchulus reniformis*. Ces deux espèces sont caractérisées par leur préférence pour des températures du sol relativement faibles et des humidités élevées; toutes deux sont capables d'entrer en anhydrobiose pendant la dessication des sols lors de la saison sèche avec des taux de survie de l'ordre de 20 à 40 %. *S. sorghi* semble strictement inféodé aux céréales sauvages et cultivées; cette espèce est, pendant la saison sèche, active et localisée en profondeur dans les sols cultivés et en anhydrobiose dans les horizons superficiels dans les sols en jachère. Seule la gamme d'hôtes relativement étroite de *R. reniformis* pourrait expliquer son absence des sols non irrigués dans la zone sahélienne ouest africaine, les autres caractéristiques écologiques étant identiques à celle de *S. sorghi*.

Key-words : *Senegalonema sorghi, Rotylenchulus reniformis,* nematode, West Africa, geographical distribution, population dynamics, vertical distribution, soil temperature, soil moisture, host plant, multiplication rate, anhydrobiosis, pathogenicity.

This eighth paper on the ecology and pathogenicity of the Hoplolaimidae (Baujard & Martiny, 1995 *b, c, d, e, f, g, h*) presents the results of field and laboratory studies on *Senegalonema sorghi* and *Rotylenchulus reniformis*.

Genera and species of the subfamily Rotylenchulinae (Hoplolaimidae) are frequently recorded in soils of West Africa : Germani (1978) listed several populations of *R. reniformis, R. parvus* and *R. borealis* from this region. Baujard and Martiny (1994) found *R. borealis* in Mali; Germani *et al.* (1984) described a new genus and a new species, *Senegalonema sorghi,* associated with sorghum roots in the centre of the peanut cropping area of Senegal. This genus has been identified recently in Mozambique around roots of sugar cane (Van den Berg, 1993). Examination of these specimens kindly sent to us by Dr. Van den Berg showed that they differ from *S. sorghi* and probably represent a new species of this rare genus.

In the peanut cropping area of Senegal, the distribution of *R. reniformis* is restricted to fields under irrigation where this species is frequent (Baujard & Martiny,

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1995 *a*). *S. sorghi* is a rare species, mostly found in the central and southern parts of this area associated with rainfed crops and wild plants (Baujard & Martiny, 1995 *a*).

Material and methods

Studies on geographical distribution, field population dynamics, and vertical distribution have been conducted as previously described (Baujard & Martiny, 1995 *b*). Unless otherwise stated, nematode extractions , nematode cultures, technics, host plants and cultivars [peanut (*Arachis hypogea* L.) cv. 55 437, millet (*pennisetum typhoides* Rich.) cv. Souna III, sorghum (*Sorghum vulgare* L.) cv. 51 69, cowpea (*Vigna unguiculata* L.) (Walp.) cv. N58 57] used for laboratory studies are those described by Baujard (1995). For the two species, inocula consisted of mixtures of vermiform stages (juveniles, immature females and males).

Origin of nematodes, laboratory stock culture conditions, and roots observations

S. sorghi : nematodes originated from soil samples taken at Nebe in an experimental field cropped with sorghum in October 1985 at the end of the rainy season. The nematodes were extracted and reared on sorghum at constant soil temperature (32 °C and soil moisture (10 %) in the laboratory until May 1992. Observations on sorghum roots infestation by *S. sorghi* were made on root systems of stock cultures stained by fuschin acid (Hooper, 1986).

R. reniformis : nematodes originated from soil samples taken at Bambey in an irrigated field cropped with vegetables in April 1990. The nematodes were extracted and reared on tomato (*Lycopersicon esculentum* L.) cv. Roma; this cultivar has been used for all laboratory experiments) at constant soil temperature (30 °C) and soil moisture (10 %) in the laboratory until May 1992.

Soil temperature

S. sorghi : tubes were inoculated with 118 ± 17 nematodes originating from 60-day-old stock cultures, planted with sorghum, and maintained at four constant soil temperature levels (30, 32, 34 or 36 °C) with seven replications for each temperature level, at 10 % constant soil moisture, for 60 days in a growth chamber with artificial lighting (16-h photoperiod).

R. reniformis : Tubes were inoculated with 117 ± 8 nematodes originating from stock cultures on tomato, planted with tomato, and maintained as mentioned above.

Soil moisture

S. sorghi : tubes were inoculated with 76 ± 5 nematodes originating from the soil temperature experiment, planted with sorghum, and maintained at four constant soil moisture levels (5, 7, 9 or 11 %) at 32 °C constant soil temperature for 60 days in a greenhouse with natural lighting. The four treatments were replicated ten times in a completely randomized design.

R. reniformis : tubes were inoculated with 140 ± 6 nematodes originating from roots extraction in the soil temperature experiment, planted with tomato, and maintained at 30 °C constant soil temperature as mentioned above.

Host plant and test for anhydrobiotic survival

S. sorghi : tubes were inoculated with 120 ± 10 nematodes originating from a 60-day-old stock culture on sorghum, planted with one of the four host plants (cowpea, millet, peanut or sorghum), and maintained at 32 °C constant soil temperature and 10 % constant soil moisture in a greenhouse. The four treatments were replicated twenty times in a completely randomized design. After 60 days, nematodes were extracted from ten replications to obtain final population counts. At this time, watering was stopped for the ten remaining replications of each treatment. These tubes were kept at 32 °C constant soil temperature and weighed daily to follow the progress of soil desiccation. Sixty days later, nematodes were extracted by elutriation.

R. reniformis : tubes were inoculated with 93 ± 10 nematodes originating from soil of the previous experiment on soil moisture, planted with one of the five host plants (cowpea, millet, peanut, sorghum or tomato), and maintained at 30 °C constant soil temperature and 10 % constant soil moisture in a greenhouse. The experiment was conducted as mentioned above.

Screening of host plants of *S. sorghi*

Tubes were inoculated with 188 ± 27 nematodes originating from 60-day-old stock cultures on sorghum and planted with one of sixteen different plants [*Andropogon guayanus* Hack., *Cenchrus biflorus* Roxb., *Eragrostis pilosa* (L.) Beauv., *Digitaria exilis* Stapf, *Panicum maximum* Jacq., *Pennisetum violaceum* L., *Zea mays* L., *Gossypium hirsutum* L. cv. Irma, *Solanum melongena* L., *Abelmoschus esculentus* ((L.) Moench, *Sesbania pachycarpa* DC., *Guiera senegalensis* J. F. Gmel., *Piliostigma reticulatum* (DC) Hochst., *Prosopis juliflora* DC., *Acacia albida* Del., *Acacia tortilis* ssp. *raddiana* Savi.] with eight replications per plant, and maintained at 32 °C constant soil temperature and 10 % constant soil moisture for 60 days in a greenhouse.

Pathogenicity of *S. sorghi* to peanut, millet, and sorghum

Separate experiments were conducted with each crop species at 32 °C soil temperature and 10 % soil moisture in a greenhouse. Nematodes originating from 60-day-old stock cultures on sorghum were inoculated onto peanut and millet at two inoculum levels (500 ± 30 or 1000 ± 60), and onto sorghum at one inoculum level

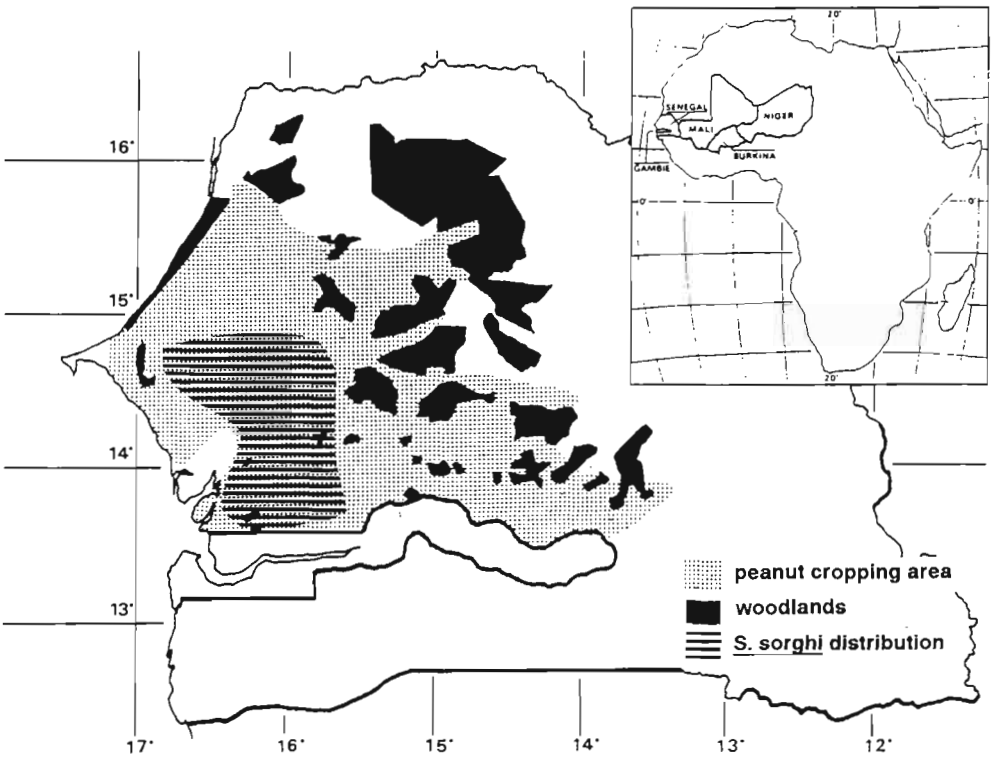

Fig. 1. *Known distribution of* Senegalonema sorghi *in the peanut cropping area of Senegal.*

(300 ± 40). Nematode effects were compared to control plants without nematodes. The treatments were replicated ten times in a completely randomized design. After 40 days, nematodes were extracted from soil and roots to determine the multiplication rate, and the fresh weight of roots and fresh and dry weights of shoots were measured.

Results

GEOGRAPHICAL DISTRIBUTION AND FIELD HOST PLANTS OF *S. SORGHI* IN SENEGAL

S. sorghi is a rare species occurring only in 7 % of 266 samples collected in the peanut cropping area of Senegal. Its distribution is restricted to the centre and the south of this area (Fig. 1). This species has been found *i)* in field cropped with peanut or millet, *ii)* in the roots of wild herbaceous *(A. guayanus)* and arborescent *(Icacina senegalensis* A. Juss., *Combretum glutinosum* Perr., *P. reticulatum)* plants, *iii)* associated with the roots of trees at depth [*A. albida* (50 cm deep), *Acacia nilotica* (L.) Willd. (from 20 to 100 cm deep), *A. tortilis* ssp. *raddiana* (from 50 to 200 cm deep), *P. juliflora* (from 20 to 60 cm deep)]. It has not been found associated with sorghum, cowpea or vegetable crops nor with *Acacia Senegal* (L.) Willd. or *Euphorbia balsamifera* Ait.

FIELD STUDIES ON *S. SORGHI*

Population dynamics

No population development was recorded in plots under peanut and cowpea monocultures and in plots under peanut-millet rotation. Small and erratic increases occurred under sorghum probably in relation with the poor growth of the crop. Significant population increases appeared only in plots under millet monoculture and permanent fallow (Fig. 2). The soil population increased from the beginning to the end of the rainy season. Nematodes invaded the plant root system with a maximum of 500 nematodes per root system. During the rainy season, population densities increased regularly over the last three years of the study (Fig. 2). At the end, or just after the end of the rainy season, population densities decreased rapidly and remained undetectable throughout the dry season (Fig. 2). Although millet appeared to be a good host for the nematode, no multiplication occurred with this crop in 1985 or in 1987 in the plots under peanut-millet rotation (Figs 2, 3). Multiplication rates during the rainy season varied from 0 to 300 and survival rates during the dry season from 0 to 50 % according to the crop and to the year (Fig. 3).

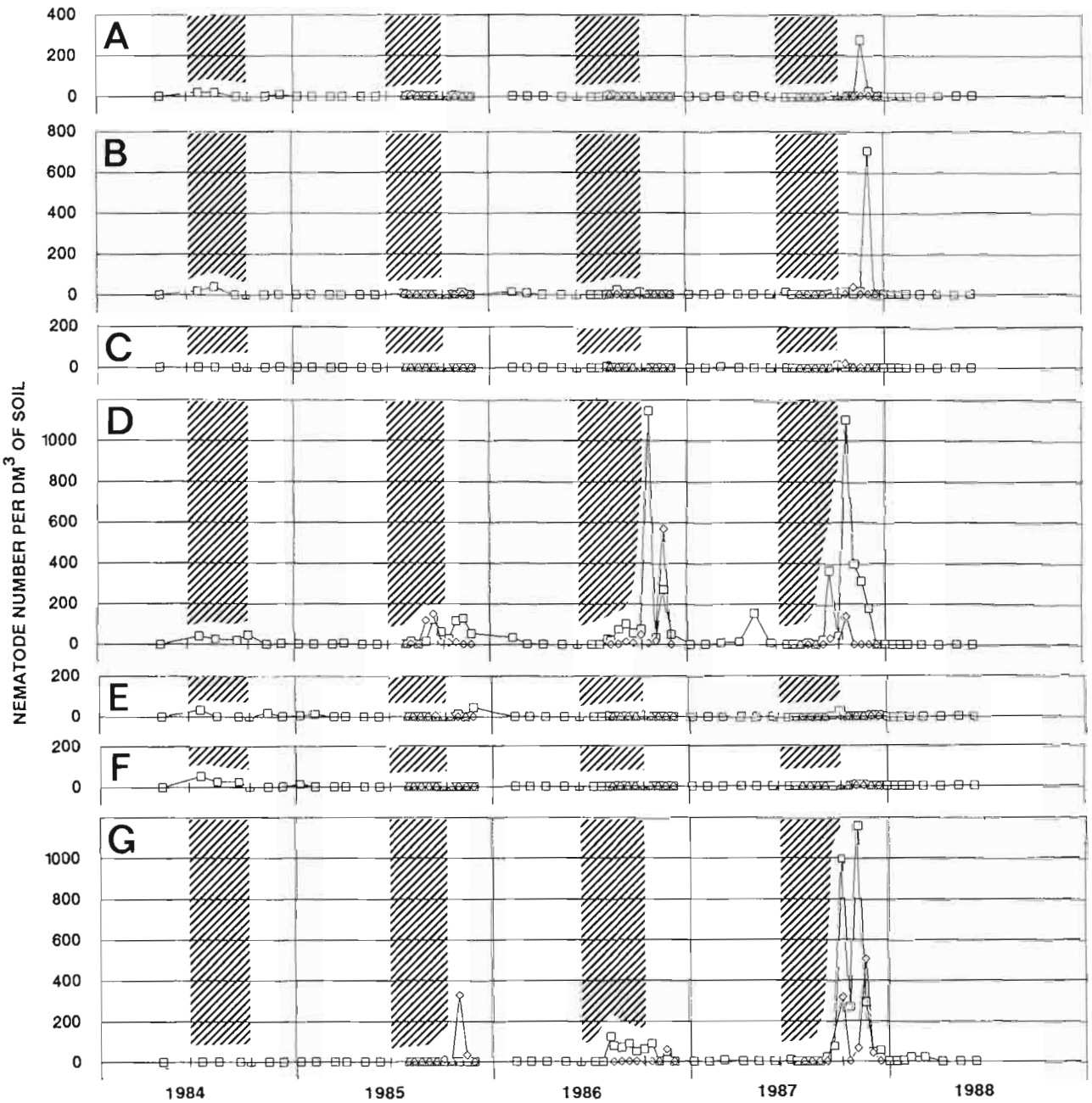

Fig. 2. *Population dynamics of* Senegalonema sorghi *according to the cultural practices. A : Peanut monoculture; B : Peanut-millet rotation without nematical treatment; C : Peanut-millet rotation with nematical treatment; D : Millet monoculture; E : Sorghum monoculture; F : Cowpea monoculture; G : Permanent fallow* (Hatched areas = rainy seasons; squares = soil population; diamonds = root population).

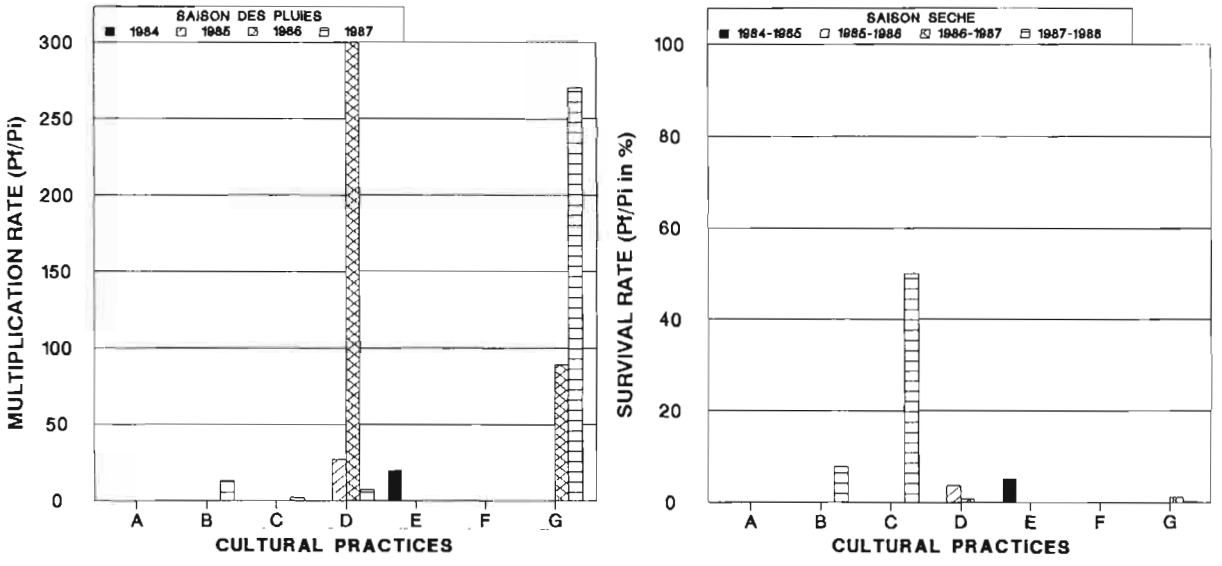

Fig. 3. Multiplication and survival rates of Senegalonema sorghi *according to the cultural practices and year of observations* (see Fig. 1 for the legend).

Vertical distribution in the soil

During the dry season, the distribution pattern of the nematode varied according to the crop. Under peanut, millet and sorghum, the nematode was located at depth, 30-35 cm below the soil surface where soil moisture never falls below 1.5 % preventing anhydrobiosis. Under fallow, the nematodes are located at a 20-25 cm depth in anhydrobiotic conditons. During the rainy season, the nematode was recovered only in the plots under millet or fallow, evenly distributed from the top to a depth of 80 cm (Fig. 4).

Laboratory studies

Observations on laboratory stock cultures of S. sorghi

All stages (juveniles, immature and mature females and males) were found in the roots of sorghum. Juveniles and immature females (Fig. 5 A) are located in the cortical cells, the head being directed to the stele. The posterior two thirds of the female body widens progressively (Fig. 5 B-D). Following this body width increase, the root tissues burst and tear so that the posterior part of the female body protrudes from the root (Fig. 5 D-F). At this stage, an ovoid shell appears around the female body (Fig. 5, F) and its volume increases following the development of the female body (Fig. 5 H-J). Eggs were laid in the cavity formed by this shell (Fig. 5 J, K). No gelatinous matrix was observed. Copulation and fertilization of females by males were not observed.

Factors affecting the multiplication rate

Soil temperature and soil moisture affected significantly the multiplication rate of both the species in the same way. Higher multiplication occurred at 30-32 °C and at the highest soil moisture levels (Figs 6, 7). Root population densities were not affected by soil temperature or moisture, 31-54 % for *S. sorghi* and 16.3-30.7 % for *R. reniformis* (Table 1). Host plants had a significant effect on the multiplication rate of both the species (Figs 6, 7). Only millet and sorghum allowed the reproduction of *S. sorghi,* whereas millet, sorghum, cowpea and tomato allowed it for *R. reniformis.* Multiplication rates of *R. reniformis* were low and variable with millet ($\times 1.25 \pm 2.13$) and sorghum (3.50 ± 1.70). Sorghum appeared to be the best host for *S. sorghi.* Root population levels remained stable. There was no relation between multiplication rates and root population densities with *R. reniformis* (Table 1).

Ability to enter anhydrobiosis

Cessation of watering induced a decrease of soil moisture down to 0.2 % in 15 days. Nematodes survived soil desiccation for 60 days, survival being related to the population densities before watering was stopped for *S. sorghi* and *R. reniformis* (Figs 6, 7). Survival rates of 20-40 % were recorded for the two species (Fig. 8).

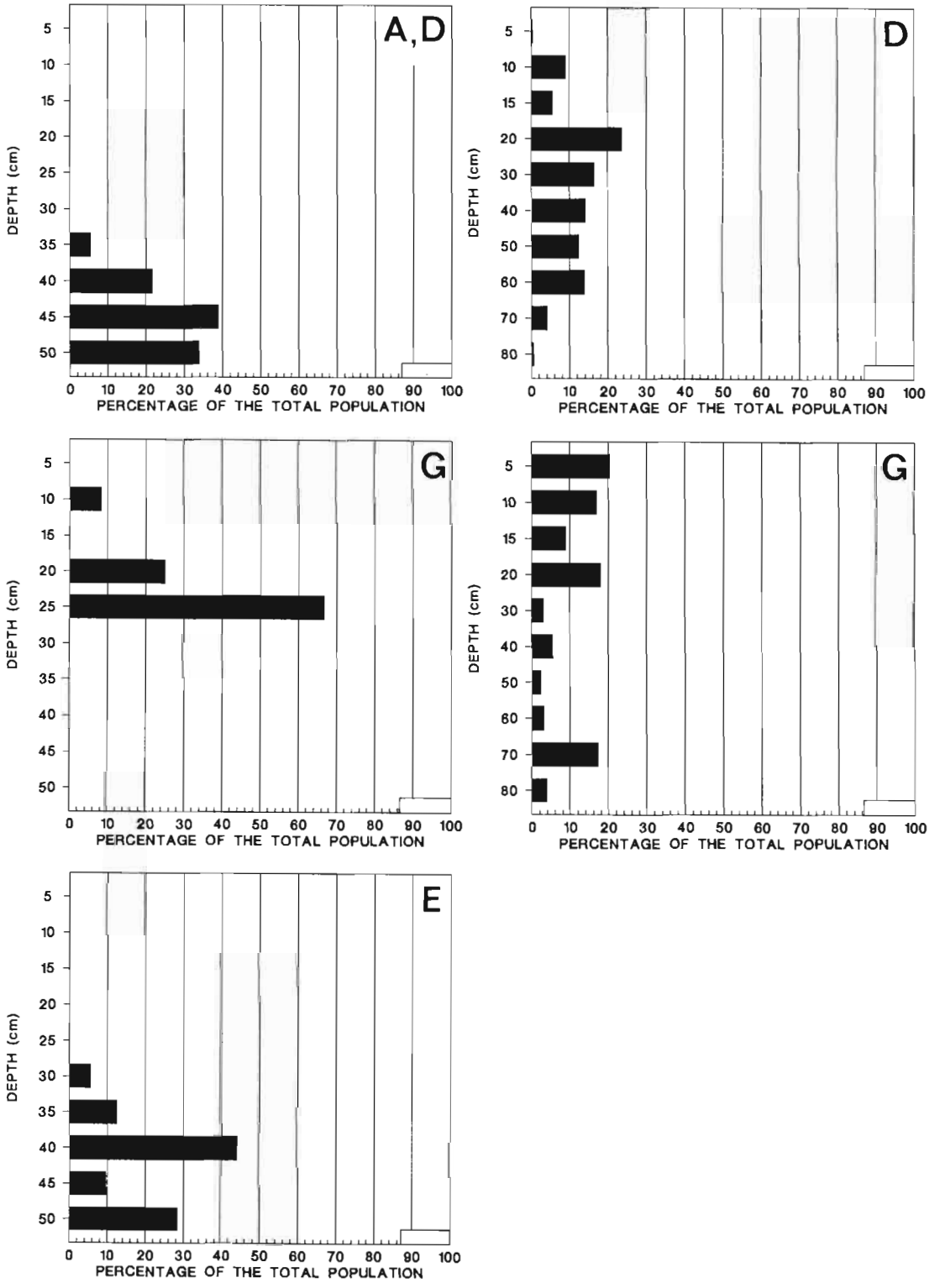

Fig. 4. *Vertical distribution of* Senegalonema sorghi *according to the cultural practices and the season of observation* (See Fig. 1 for the legend; left column : dry season; right column : rainy season).

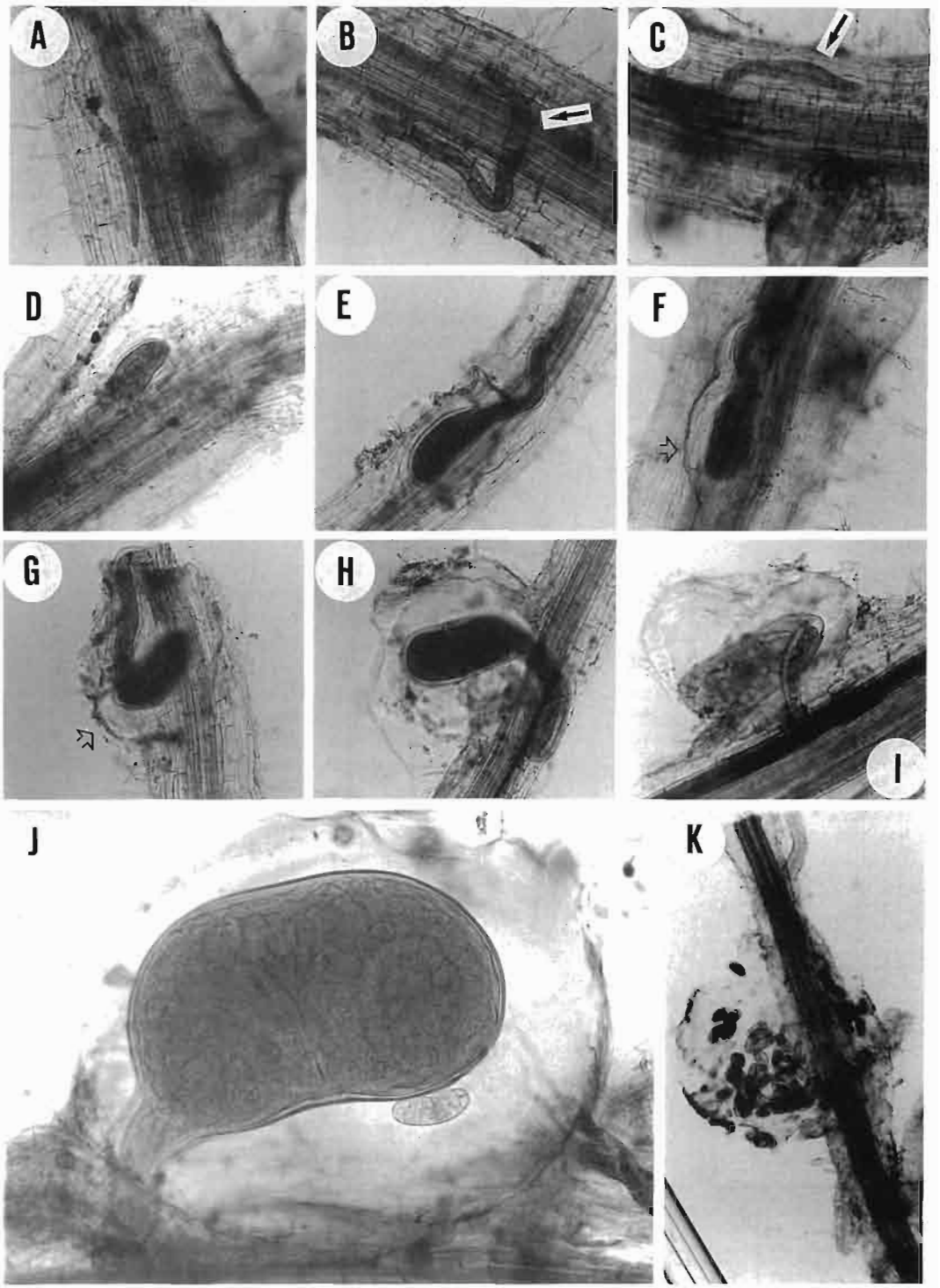

Fig. 5. *Development of* Senegalonema sorghi *in the roots of sorghum. A : Immature female in the parenchyma; B-E : Swelling (arrows) of the posterior part of the body; F-G : Formation of the shell (arrows) around the body and progress of body swelling; H-J : End of the swelling process; J-I : Egg laying without gelatinous matrix.*

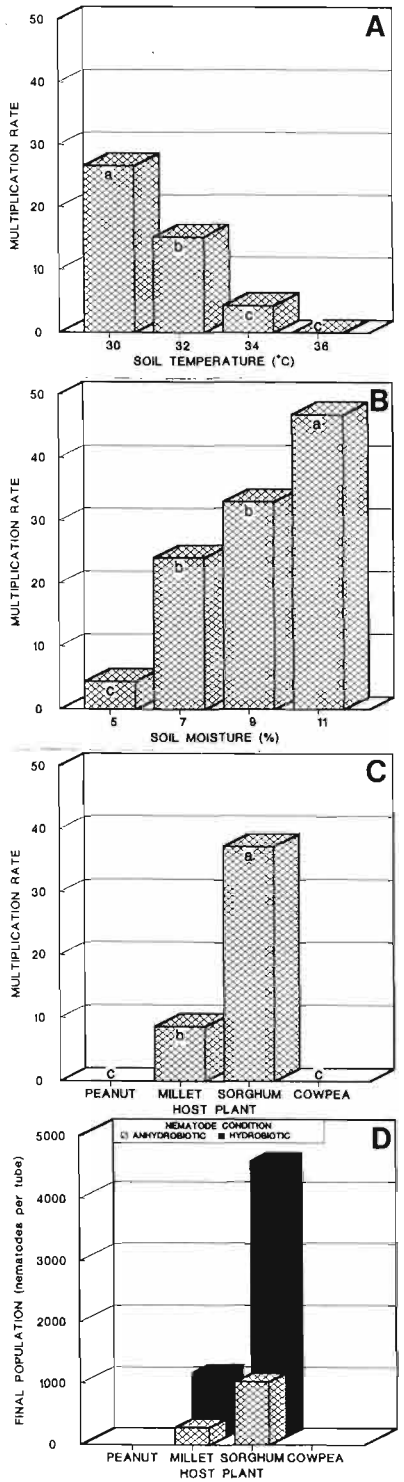

Fig. 6. *Effects of soil temperature (A), soil moisture (B), and host plants (C), on the multiplication rate and effect of soil desiccation on survival (D) of* Senegalonema sorghi (In each experiment, data followed by the same letter are not significantly different at $P < 0.05$).

Screening of host plants of S. sorghi

All the graminaceous plants tested were hosts for the nematode with multiplication rates varying from 3 to 32 : *P. violaceum* (× 2.8), *Z. mays* (× 8.5), *A. guayanus* (× 8.8), *P. maximum* (× 16.4), *C. biflorus* (× 20.9), *E. pilosa* (× 27.7), *D. exilis* (31.9). The other plants tested did not allow the multiplication of the nematode and no nematodes were recovered from the root systems.

Pathogenicity

S. sorghi did not have any significant effect on growth of peanut at the inoculum levels tested. The nematode significantly reduced the root development of millet, and reduced dry weights of roots and shoots of sorghum (Table 2). Root population levels varied as in the previous experiments (Table 1).

Discussion

Field observations showed that *S. sorghi* is characterized by *i)* its limited geographical distribution, *ii)* its host range being restricted to wild and cropped cereals, *iii)* its capacity to survive in active stage at deeper soil levels, or under anhydrobiosis in the upper layers of the soil during the dry season. Laboratory experiments confirmed these observations, showing that this nematode is not well adapted to the ecological characteristics of the semi-arid areas of West Africa. Relatively low soil temperatures and high soil moisture levels are required for optimal development of this species. But the nematode is able to enter anhydrobiosis and it feeds only on graminaceous plants including cereals. These characteristics and the distribution of the nematode in deeper soil areas in croped soil might explain its low frequency of detection during nematological surveys.

R. reniformis exhibited the same ecological characteristics as *S. sorghi* for optimal soil temperature and moisture. Previous studies have shown that cereals are non-hosts or poor hosts for this species (Luc & de Guiran, 1960; Caveness, 1967; Stoyanov, 1967) and these characteristics might explain the limited distribution of this species to vegetable crops under irrigation in the semi-arid tropics of West Africa.

The shell embedding the mature female of *S. sorghi* in the roots of sorghum constitutes a new biological characteristic of swollen mature females in the Tylenchina. Recent ultrastructural observations showed that this shell is probably of polysaccharidic composition (Mounport, pers. comm.); it appears similar to that described in the genus *Achlysiella* (Hunt *et al.*, 1989). The biology of *S. sorghi* differs from that of *R. reniformis* by *i)* the presence of a shell around the mature female, *ii)* the absence of a gelatinous matrix, *iii)* the presence of all the nematode stages (*vs* only females) in the roots (Linford & Oliveira, 1940; Sivakumar & Seshadri, 1971), *iv)* low (× 20-50) *vs* high (1000-2000) multiplication rates.

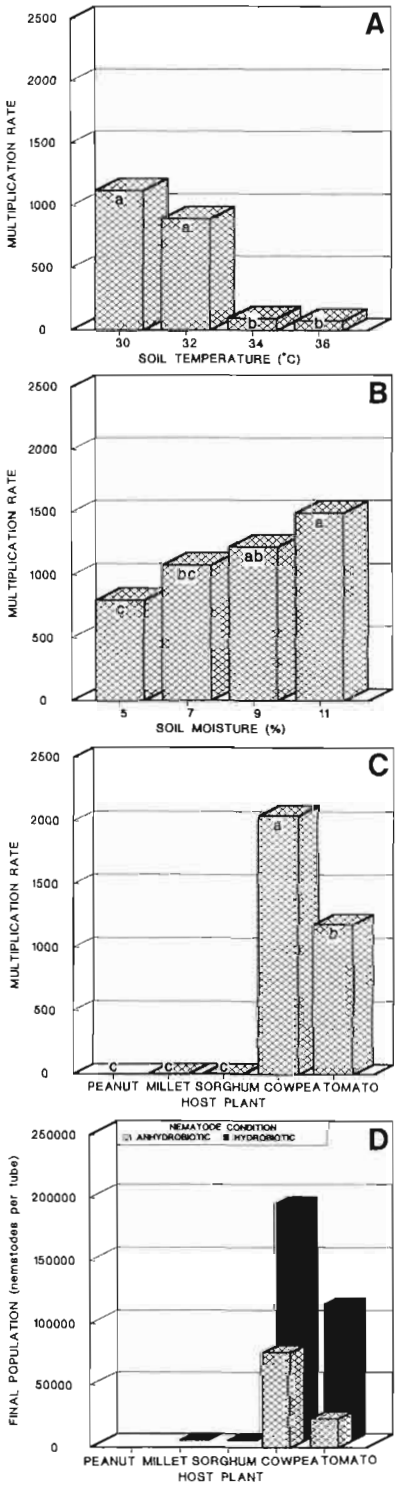

***Fig.** 7. Effects of soil temperature (A), soil moisture (B) and host plants (C) on the multiplication rate and effects of soil desiccation on survival of* Rotylenchulus reniformis (In each experiment, data followed by the same letter are not significantly different at $P < 0.05$).

***Table 1.** Percentages of the population of* Senegalonema sorghi *and* Rotylenchulus reniformis *in the roots at the end of the different experiments on multiplication rate (ND : not determined).*

Experiment	Treatment as a % of total	Root population tube population	
		S. sorghi	*R. reniformis*
Soil temperature	30 °C	31.6	26.9
	32 °C	47.2	27.9
	34 °C	48.3	30.7
	36 °C	35.2	25.1
Soil moisture	5 %	33.8	20.1
	7 %	54.2	16.9
	9 %	54.3	16.9
	11 %	48.3	16.3
Host plants	peanut	0	0
	millet	33.1	37.5
	sorghum	40.5	45.9
	cowpea	0	36.0
	tomato	ND	30.0
Pathogenicity			
Peanut			
* 500 nematodes		0	ND
*1000 nematodes		0	ND
Millet			
* 500 nematodes		60.5	ND
*1000 nematodes		59.3	ND
Sorghum			
* 300 nematodes		62.1	ND

* inoculum pet tube.

***Table 2.** Multiplication rate and effects of* Senegalonema sorghi *on peanut, millet, and sorghum (numbers followed by the same letter are not significantly different at* $P < 0.05$*).*

Plant	Inoculum	Multiplication rate	Fresh weight (g)		Dry weight (g)
			Roots	Shoots	Shoots
Peanut	0	–	1.72 *a*	6.63 *a*	1.15 *a*
	500	0.03	1.88 *a*	6.55 *a*	1.11 *a*
	1000	0.03	1.71 *a*	6.56 *a*	1.12 *a*
Millet	0	–	2.16 *a*	4.62 *a*	0.74 *a*
	500	3.91	1.76 *ab*	4.46 *a*	0.63 *a*
	1000	2.72	1.31 *a*	3.57 *a*	0.50 *a*
Sorghum	0	–	2.22 *a*	4.33 *a*	0.66 *a*
	300	3.29	1.58 *b*	3.74 *a*	0.53 *b*

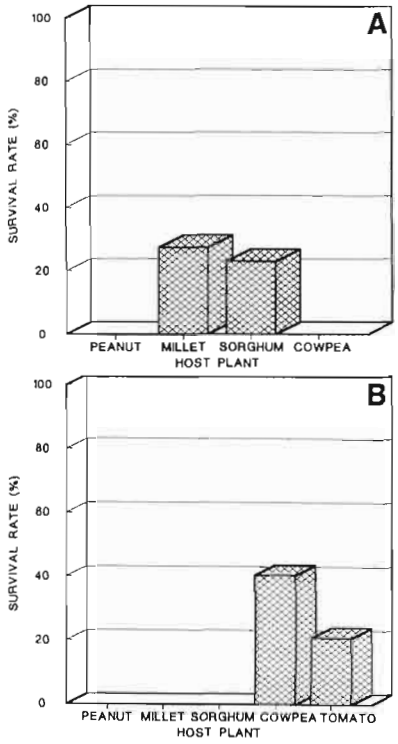

Fig. 8. *Effects of host plants and soil desiccation on survival rate* Senegalonema sorghi *(A) and* Rotylenchulus reniformis *(B).*

Acknowledgments

The authors thank Dr. E. van den Berg for sending specimens of *Senegalonema* sp. from Mozambique.

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